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Monograph: *In vitro* efficacy of 30 ethnomedicinal plants used by Indian aborigines against 6 multidrug resistant Gram-positive pathogenic bacteria

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PEER REVIEW

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Comments

Methodology is universally accepted. Data were well presented. Since plant extracts have an array of compounds, the control of MDR bacteria was easy. Thus, plant extracts could be used as CAM. These 30 plants are used by Odishan ethnic tribes as traditional medicine.

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ABSTRACT

Objective: To monitor *in vitro* antibacterial activities of leaf extracts of 30 common and non-common plants used by aborigines in Kalahandi district, Odisha, against 6 clinically isolated multidrug resistant (MDR) Gram-positive bacteria of 3 genera, *Staphylococcus*, *Streptococcus*, and *Enterococcus*.

Methods: The antibiotic sensitivity patterns of 6 bacterial strains were studied with the disk-diffusion method with 17 antibiotics belonging to 8 classes. Monitored plants have ethno-medicinal use and several are used as traditional medicines. Antibacterial properties were studied with the agar-well diffusion method. Minimum inhibitory concentration and minimum bactericidal concentration values of ethanolic and aqueous extracts of plants were determined by the microbroth-dilution method.

Results: Ethanolic plant-extracts had the better antibacterial potencies in comparison to their corresponding aqueous extracts. Plants with most conspicuous antibacterial properties in controlling MDR strains of Gram-positive bacteria were aqueous and ethanolic extracts of plants, *Ixora coccinea*, *Nyctanthes arbor-tristis*, *Polycythaemia rubra*, *Pongamia pinnata* and *Syzygium cumini*, *Carthamus tinctorius*, *Cucurbita maxima*, *Murraya koenigii*, *Leucas aspera*, *Plumbago indica* and *Psidium guajava*. Ethanolic extracts of most plants had phytochemicals, alkaloids, glycosides, terpenoids, reducing sugars, saponins, tannins, flavonoids and steroids.

Conclusions: These plants could be used further for the isolation of pure compounds to be used as complementary non-microbial antimicrobial medicines.

KEYWORDS

Ethnobotany, Antibacterial property, MDR bacteria, Phyto-extracts, Phytochemical analyses, Minimum bactericidal concentration

1. Introduction

A vast amount of literature has accumulated on the emergence and associated artifices of multidrug resistant (MDR) strains of all most all pathogenic bacteria[1]. It would be a mistake if alternate strategies for the control of MDR pathogens were not seriously pursued, as the development of dovetailed drugs from the mainstream medicine is comparatively slow. MDR pathogens often enforce patients to hospice at younger ages even, due to intractable infections at innards, leading to the slow onset of a terminal illness, that gruels emotionally till death. As the subject of the use of herbal drugs was suggested suitably in developed countries[2], corroborated by World Health Organization[3], the development of alternate therapy for the control of MDR bacteria is considered as the urgent need for apothecary (pharmacognosy, pharmacology and pharmaceutics)

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who must prudently take up the associated endeavour. Thus, it is never a matter of choice rather than a matter of compulsion to lean to phyto-drugs, as those have been serving the humanity from the time immemorial. The modern medicinal system has several phyto-drugs in use such as, morphine, quinine, vinblastin, vincristine, atropine, digoxin, and taxol, to name a few in brief. Today, plants provide a 25% drugs prescribed worldwide, and 121 active compounds are in the current use for chemoprophylaxis. In addition, WHO recognizes 252 basic and essential drugs; an 11% drugs are exclusively from plant origin; a 60% of anti-tumor and anti-infectious drugs from natural origin are under clinical trials[3]. Thus, phyto-drugs gave good sense of promise to apothecary, from the vantage point of the inherent plethora of natural chemicals. Obviously, a vast majority of these drugs cannot be synthesized economically, and are obtained from wild and cultivated plant sources only. Further, a lot of pure phytocompounds have been developed and are used as drugs paradigmatically, by their own merit today[4]. By the by, the use of crude plant extracts is remarkably popular in consumerism. Furthermore, the primary benefits of plant-based medicines are comparatively sought after in tide of love for natural products rather than the synthetic ones, which have been well discussed in tubercle bacilli (TB) chemotherapy, elsewhere[5]. It is consensus that most plants have the history of traditional use as ethnomedicine; nevertheless, certain phytochemicals particularly from non-edible/poisonous plants have unknown and known toxic effects that need be quantified pharmacologically before being used as drugs.

Indeed, drug resistance in pathogenic bacteria has been a commonplace of infection biology, for both Gram-negative (GN) and Gram-positive (GP) ones[6,7]. Virulent enteric bacteria, *Klebsiella, Salmonella, Pseudomonas, Shigella, Escherichia* and a few more are active in unhygienic, marginalized communities and urban-slum ghettos in developing countries on precipitating public health episodes[8]. In parallel several GP bacteria, MDR species of *Staphylococcus*, *Enterococcus* and *Streptococcus* have also created frightening situations due to the development of resistance to β-lactams and several other groups of antibiotics. For example, the methicillin resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) had emerged with resistant to 95% of presently used antibiotics worldwide, and this particular bacterium, once known as a commensal inhabiting soft zones of human body, is now regarded as a superbug in the health domain for its unbridled notorious standard of virulence. Indeed, its evolved strains have the multidrug resistance character by acquiring complicated acquired/mutational clonal nexuses. MRSA, vancomycin resistant *S. aureus* (VRSA), vancomycin intermediate *S. aureus* (VISA) have been the major causative organisms of morbidity and mortality due to acute surgical site infections and wound suppurations, everywhere. Particularly, the emergence of MRSA had led to the use of alternative drug, the clindamycin (macrolide) with blithesome control. However, the prevalence of the clindamycin resistant MRSA strain had also been reported from many laboratories, impeding smooth clinical management[9]. Inducible clindamycin resistance in MRSA, due to erythromycin resistance was reported from this hospital[10], challenging the hygienic condition of intensive care units (ICUs)[11]. Moreover, strains of *S. aureus* (MRSA, VISA and VRSA) have been prevalent at 20.83% in our hospital over a period of 30 months, ending in April 2012. Similarly, vancomycin resistant Enterococci (VRE) strains since 1982 till date worldwide have been slowly taken up a noteworthy momentum of spread, and VRE is regarded as the second-most prevalent GP pathogen in nosocomial settings[10, 12]. In our hospital as well, VRE and vancomycin sensitive Enterococci (VSE) strains were seen to be present in equal proportions, over a period of surveillance of 6 months[13]. Species of *Enterococcus* [*Enterococcus faecalis* (*E. faecalis*), *Enterococcus avium, Enterococcus durans, Enterococcus faecium, Enterococcus gallinarum* and *Enterococcus solitarius*] are primarily opportunistic pathogens and the intensive use of broad spectrum antibiotics has been attributed to the emergence of their MDR strains. For example, during surveillance in hospitals of the USA from 1999 to 2002, it was recorded that 9% of nosocomial blood stream infections were due to Enterococci; of them, 60% strains were VRE[14]. Most frequently, VRE colonizes gastrointestinal tract and skin. Incidentally, hospitalized patients and non-hospitalized workers as controls in a cattle-rearing area of France were known to have the asymptomatic carriage of VRE[15]. Further, many antimicrobials used for small animals (pets and food animals) are used in humans that promotes the transmission of bacteria to their owners. For example, drug-resistant strains of *Staphylococcus intermedius, Campylobacter* sp., *Salmonella* sp. and *Escherichia coli* (*E. coli*) had been cited as possible zoonotic concerns[16]. In dogs, *E. coli* strains were phylogenetically similar to pathogenic strains causing infection in human beings; more than 15% of canine fecal deposits in the environment contain *E. coli* strains related to virulent human strains, asymptomatically[17]. Obviously, asymptomatic carriage of pathogenic bacteria may lead to the risk of development of severe infections in due course of time when temporarily the body has a setback in the defense mechanism. *Enterococcus* sp. causes bacteremia, endocarditis, meningitis and diverticulitis[18]. Additionally, avoparcin, a glycopeptide antibiotic is used as a growth promoter in animal farming in Europe, and consequently avoparcin induced VRE had been prevalent. Surprisingly, one would hardly find a more vivid illustration of a commensal, transforming into a perilous pathogen with an armamentarium of multidrug resistance, in the last few decades, as *S. aureus* or *E. coli* is.

The aim of this work was to verify 30 common and non-common plants used by aborigines of Odisha, in an attempt to identify their control over 6 MDR GP pathogenic bacterial strains *in vitro*. Antibiograms of those isolated bacteria with 17 antibiotics of the day ascertained that all were amply MDR. Thus, work on individual plants in controlling MDR strains of bacteria was recorded. Values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) with phyto-extracts of MDR bacteria had been recorded. All these plants have ethnomedicinal uses and many of them are used as complementary alternate medicine (CAM). The recorded

data are anticipated to trigger work on the isolation of pure compounds for further scientific use in the crusade of the control of MDR pathogens. This is a record of scientific verification of ethnomedicinal information on a group of plants from Kalahandi forest, in continuation to the previous work[19], and for the possible use of these plants as CAM.

1.1. Literature survey of pathogenic bacteria

Frequent reports of resistance to the β-lactam group of antibiotics on GN bacteria comprising, enteropathogens, uropathogens and suppuratives have created a vacuum in the confidence of clinicians[20]. Sometimes, ICUs of hospitals have become cesspool-like dangerous places, since a patient admitted for one cause may often go back to community (society) with some newly acquired infectious pathogenic bacterium, which will cause to spread bacterium in community. This might well demonstrate in the surveillance of GN [*Acinetobacter baumannii* (*A. baumannii*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae*] and GP (MRSA and MDR strains of Enterococci) bacteria[6, 21]. Eventually, the empirical use of antibiotics intended frequently for several infections becomes ineffective. As antibiotics have a pivotal role in clinical management today, it would be hard to think of an aspect of contemporary life that does not depend on antibiotics. Moreover, with a smattering of well-heeled and rich people in developing countries, the rise of clinical costs due to ailments from MDR bacteria can never be appropriated, as those have proved as notorious communal and nosocomial pathogens with diverse armamentaria of drug resistance[22].

Streptococci are another group of commensals; α-haemolytic species cause pneumonia and *Streptococcus mutans* (*S. mutans*) causes dental caries and endocarditis. The most common pathogen, *Streptococcus pyogenes* (*S. pyogenes*) (Group A streptococci) is a β-haemolytic species causing suppurative infections, streptococcal pharyngititis and scarlet fever. *Streptococcus agalactiae* (Group B streptococci) causes pneumonia, meningitis and bacteremia in neonates as well as in aged people with occasional systemic infections. These enteropathogens also infect the female genital tract, and cause premature rupture of membranes (PROM) during pregnancy and consequent neonatal infection ending in septicemia[23]. The γ-haemolytic strain of *Streptococcus pneumoniae* cause bacterial pneumonia, otitis media and sometimes leads to meningitis, and causes mortality in almost 1.6 million people every year, worldwide[24]. In the last 3 decades, resistance patterns of *Streptococcus pneumoniae* to β-lactams and macrolides have increased dramatically, worldwide[25].

Ferocious pandrug resistant (resistant bacteria to all antibiotics of the present time) are *A. baumannii*, *Klebsiella pneumoniae*, *P. aeruginosa*[26]. To control these pandrug resistant GN bacteria, a synergistic or combination therapy is often used with colistin (polymixin) and one from an older class of antibiotics, piperacillin or tazobactam, till date. In a study from Darjeeling (India), it was recorded that *E. coli* strains were resistant to almost all major antibiotics in present day; 20 antibiotics of different groups comprising three third-generation cephalosporins were used in the study[27].

Further, under-5 child mortality from shigellosis had been estimated almost 99% of 165 million annual episodes[28], notwithstanding, governmental measures for the prevention and control of such fervent episodes. This grievous situation is frequently seen in elderly and immunocompromised people in the developing nations. Not surprisingly, empiric therapy with the first line antimicrobial drugs, ampicillin, trimethoprim/ sulfamethoxazole, chloramphenicol, nalidixic acid, co-trimoxazole and tetracycline against several infections were progressively ineffective due to bacterial multidrug resistance in developed nations[29,30]. For MDR *Shigella* sp., the most preferred therapeutic options are fluoroquinolones for adults, oxyimino-cephalosporins for children. However in this study, resistance of 4 species of *Shigella* to ciprofloxacin were in the range 35% to 42% of isolated strains, for gatifloxacin, the resistance value was 28% to 78%, for levofloxacin, it was 28 -51%, for ofloxacin the value was 19 to 41%, for clinically isolated strains. Thus, the failure of fluoroquinolones created an average chance of 30% to 35% in the empiric therapy for *Shigella* sp. Of the three β-lactams, aztreonam was resistant to all the four *Shigella* sp. from 18% to 23%. Similarly, piperacillin/tazobactam was resistant by 18% to 31% while piperacillin was resistant by 78% to 88% of *Shigella* strains. Additionally, *Shigella* had extended spectrum β-lactamase (ESBL) producing clinical isolates in our study. Moreover, *Shigella sonnei* was reported to have the β-lactamase production by the plasmid mediation. *Shigella sonnei* had also been known as the predominant causative organism of enteric diseases in Asia, *a priory* for its production of chimeric β-lactamases, with the gene CTX-M-64[31]. Furthermore, quinolone-resistant *E. coli* strains were reported as widespread in Asia[32].

Studies on antibiotic-resistant mutants of enteropathogenic strains from Barcelona revealed that *Campylobacter* was the leading MDR pathogen, followed by *Salmonella*, *Shigella* and *Yersinia*[33]. In fact, *Campylobacter* and *Salmonella* are known to cause extra-intestinal pathogenesis, the latter causing UTI and abscess at various body parts-all leading to bacteremia. From an Indian study, it was reported that *Shigella*, *Salmonella*, *Aeromonas* sp. and *Vibrio cholerae* (*V. cholerae*) were the most frequently isolated pathogens from stools of under-5 children with diarrhoea. *Shigella* was the most frequent pathogen, while non-typhoidal *Salmonella* and *V. cholerae* were in the decreasing trend of prevalence. Nalidixic acid, co-trimoxazole and furazolidone were resistant to *Shigella*, while *V. cholerae* strains were resistant to nalidixic acid and co-trimoxazole[34]. *Shigella*, *Salmonella* and *E. coli* were the most frequently isolated enteric pathogens in children of Ghana, among which, the majority isolates of *E. coli* and *Shigella* were resistant to methicillin, trimethoprim/ sulfamethoxazole and chloramphenicol[35].

The emergence of MDR strains of pathogens has many routes-drug efflux mechanisms operative at the plasma membrane level that is well demonstrated in *E. coli* and other pathogenic bacteria[36]. Secondly, extracellular and intracellular degradations of antibiotics are epitomized

by ESBL and carbapenamase producing GN bacteria; but the development of drug resistance has not been properly perceived. Thirdly, the drug resistant character probably in R-plasmids or transposons are transferred from one bacterium to the other; the receiving bacterium may be even from a distant phylogeny; for example, the transfer of the multiple antibiotic resistance locus from *E. coli* had been demonstrated to be active in *Mycobacterium smegmatis*[36].

Antibiotic sensitive pathogens have a limited capacity of virulence as the employed antibiotic controls them *in vivo*. At a particular level, the host defense system also helps control of pathogens, when the later are in a smattering number. Indeed, for the internal protection, antibiotic producing organisms harbour antibiotic resistant genes in plasmids and chromosomes as well as the associated transfer mechanisms[37,38]. Therefore, such genes and/or transposon must have been taken up, *a priory*, horizontally by the susceptible group of bacteria, via bacterial transformation and/or conjugation[39,40].

Moreover, bacteria having simple/ plastic genomes undergo intrinsic (mutations) or acquire genetic (conjugations and transformation) changes in the presence of an antibiotic, as a stress factor from a drug resistant strain. As a result, accrual antibiotic resistance mechanisms are the clinical determinants of the pathogenesis. Indeed, the horizontal transfer of genetic materials from one organism to another appears faster than mutational changes, a phenomenon popularly called as 'evolution of quantum leaps'[41]. Slowly, the use of more and more antibiotics for the control of infectious diseases, have led to multiple resistances, *i.e.*, too many antibiotics are ineffective to progressively increasing resistant strains of pathogens, as if growth and momentum gained by a descending snow-ball, during the passage of time by mutation and acquisition of genes from related/ unrelated bacteria, ending in shockingly repellant multidrug resistance. Older antibiotics slowly become obsolete. Therefore, those antibiotics were never applied. Drug resistant bacteria gain the capability of surviving and multiplying under antibiotic-stress conditions, confirming the biological rule: 'any limiting condition for the majority would be an excellent opportunity for the minority'. In the presence of a drug in a body *in vivo*, the progeny of a drug sensitive strain is eliminated and the resistant strain survives, multiplies as if developing from a doppelgänger, and predominates ultimately in causing a characteristic pathogenesis. It is the reason why a suitable emulating agent for the control is absent, and if plant-based CAM were present in parallel along with the employed antibiotic, there would be the coveted blithesome result.

Moreover, the expression of antibiotic resistance in a pathogen is co-expressed with virulence, demonstrated in *E. coli* with the mar-locus (multiple antibiotic resistance locus) that regulates the expression of 60 chromosomal genes, along with the expression of multidrug resistance. The resultant clinical consternation is the linkage between antibiotic resistance and the expression of virulent gene regulation. It had been demonstrated that antibiotic susceptibility of bacteria is modulated by several factors, growth phase, pH, carbon dioxide concentration, temperature, salt concentration, and low iron content[42]. For example, in *P. aeruginosa*, the quinolone treatment at a sub-inhibitory level induced the expression of certain bacterial genes, which probably should be expressive in quinolone-resistant strains of the bacterium. Similarly, ciprofloxacin causes the Shiga-toxin production by *E. coli* O157:H7 both *in vitro* and in an animal model[43]. Thus, antibiotic stress induces the expression of virulence at sub-lethal levels of the challenging antibiotic. In such a situation, the use of a combination therapy, with cognitively skilled with 'two antibiotics and two drugs' at levels far below the mutant preventive concentrations, at which those were non-toxic to host, proved dangerous with *Mycobacterium tuberculosis* by emergence of its MDR strains[5]. In addition, it had been proved that quinolone-resistant *S. aureus*, when treated with a sub-inhibitory concentration of a quinolone increased the expression of fibronectin-binding proteins contributing to the emergence of the virulent factor[44]. It was demonstrated that the antibiotic treatment triggered the release of bacterial products including endotoxin and lipopolysaccharide, which improved its virulence character, and of related pathogenic bacteria[45]. In addition, an exposure to low concentration of antibiotics triggered the sigma factor, which was linked to virulence, demonstrated in TB[46]. Therefore, the effect of antibiotics in a mixture of drug-resistant and -sensitive pathogens is a complex matter inducing several target and non-target effects. For example, isoniazid-induced alterations in TB genome had been recorded[47]. In such a situation, an application of an antibiotic to a pathogen resistant to another antibiotic of the same class of antibiotic promotes epidemics, since the absence of a suitable/emulating control agent. A synergistic use of CAM could be helpful in the situation.

MDR bacteria could be taken as if, the return of an enemy with extra strength (multiple resistance) after an earlier half-hurt by an antibiotic. Defenses produced by the host body sometimes are counteracted by the MDR marauding pathogen, as successful parasites live and reproduce to live-multiply for affecting pathogenesis. This has been demonstrated with *Salmonella enterica* serotype typhimurium[48,49]. Even, MDR *Neisseria gonorrhea* had been known to acquire multiple transferrable resistance regulator (mtrR) and sensitivity to antimicrobial peptides A (SAP A) type MDR systems of genes, from *Salmonella enterica* serotype typhimurium[50].

β-lactam antibiotics are widely used as antibacterial agents. When *S. aureus* was able to produce penicillinase before 1960 after its introduction in 1940, methicillin was introduced to combat penicillinase producing *S. aureus* and MRSA was reported in 1961 itself[51]. Today, MRSA is the most infamous GP pathogen of hospitals everywhere. Methicillin resistance has arisen by the acquisition of a novel DNA stretch, which results in the production of newer penicillin binding proteins (PBP) namely, PBP2′ or PBP2a. In the meantime, around 2003, cephalosporins were developed to control MRSA. PBP2′ is a developed protein and it is the product of 0.2 kb *mecA* gene; this is a part of the larger mobile genetic elements in Staphylococci, the staphylococcal chromosomal cassette mec. Around 5 different staphylococcal chromosomal cassette mec types

have been described, which vary in size from 0.2 kb to 0.7 kb[52]. Further, apart from the *mecA* gene, complex genetic elements may contain integrated plasmids and transposons confirming resistance to other antibiotic classes. In fact, penicillinase production is common in *S. aureus*, but was rare in Enterococci, two decades ago[53], and up to that time it was not reported in other Streptococci even. Indeed, Enterococci are intrinsically resistant to marketed/available cephalosporins, as PBP has low affinity to cephalosporins for causing the degradation.

Transferable (vancomycin) glycopeptide resistance was first reported with the recognition of Enterococci with the gene *VanA*[54]. Other variants of glycopeptide resistant Enterococci (GRE) with *VanB*, *VanC*, *VanD*, *VanE* and *VanG* were reported subsequently[55]. GREs of all these types remain however, susceptible to all types of novel glycopeptides. Glycopeptides have a key role in serious infections in MRSA too. But, the development of VRSA and VISA had been recorded progressively leading to the spread of VRSA, worldwide. Teicoplanin (glycopeptide) was in use for the control of VISA/VRSA. But, both these glycopeptides were resistant to the present GP isolates. Moreover, Streptococci and Enterococci were insusceptible to gentamicin and other aminoglycosides due to poor transport across cytoplasmic membranes, a synergistic combination of derivatives of penicillin and aminoglycoside was used against these two pathogens elsewhere[56]. But, our isolates were amply resistant to two used aminoglycosides. Moreover, numerous mechanisms have been known for the resistance of GP bacteria to macrolides, lincosamides and streptogramin B agents (MLSB group)[57]. The MLSB group becomes resistant to MRSA by 23s rRNA methylases encoded by *erm* genes[7]. Efflux pumps are the major facilitators in the mechanism of macrolide resistance mediated by *mef* genes operated in Staphylococci. Other than *erm* and *mef* genes, a specific resistant pattern to lincosamides was reported in outlandish alleles of Staphylococci[57]. Further, fluoroquinolones target the resistance at the DNA-gyrase gene. Ciprofloxacin resistance has been shown to be mediated by both efflux mechanism and mutational target modifications. And the pumps involved in resistance in *S. aureus* are due to *nor* genes[58]. Thus the infection dynamics of MDR pathogens challenges the hygienic totem pole of a country; herein the artifices of 'totally drug resistant TB', reported from Italy and India are not considered. This clearly demonstrates the chicaning characteristics of MDR incarnations of pathogenic bacteria that need be controlled with an iron hand, and phytodrugs always remain as an affordable source of non-microbial antimicrobials, which are not yet adopted by the antimicrobial stewardship formally, despite the alarming situation of pathogen induced episodes in all hospitals, everywhere.

2. Materials and methods

2.1. Survey work

Plants reported (listed in Table 1, Figures 1-18) were collected from Kalahandi forest during December 2009; 15 hamlets (villages) of Junagarh block of Kalahandi district were surveyed. Junagarh is situated at 19°10′ and 20°30′ north latitude and 82°30′ and 83°50′ east longitude. The elevation ranges from 400 to 1 200 m; temperature varies from 10 °C in winter to 46 °C in summer and the district experiences an average rain fall of about 128 cm and a rich biodiversity, typical to a sub-tropical forest. The survey was done with a questioner and personal interview, using the snowball technique in survey and sampling[19].

2.2. Preparation of plant extracts

A lot of 20 g of powder from clean leaf-samples was dissolved separately in aliquots of 200 mL sterile double-distilled water and 200 mL 80% ethanol, in wide-mouth bottles and bottles were incubated at room temperature for 48 h. Each mixture was hand-shaken at every 3-4 h, and filtered; filtrates were concentrated with the help of a rotary evaporator at 40 °C, till sticky masses were obtained, and those were incubated in a desiccator with fused calcium chloride for hardening sticky masses. These steps were repeated for each plant sample: 0.02 to 1.4 g solid sticky mass/ 20 g leaf-powders of each plant was obtained, dissolved in 2 mL aliquots of 10% dimethyl sulfoxide (DMSO) and was stored at 4 °C until further use.

2.3. Phytochemical analysis of plants

Plant extracts using ethanol and water were subjected to several chemical tests to know the presence of flavonoids, saponins, phlobatannis, resins, sterols, lipids/ fats, steroids, tannins, glycosides, acidic compounds, terpenoids, reducing sugar, phenols, carbohydrates and anthra-quinones[59,60].

2.4. Isolation of bacteria from clinical samples

Nutrient broth (NB) and nutrient agar (NA) (HiMedia, Mumbai) were used for bacterial growth. The bacterium intended for Gram-staining was in the log phase of growth. The test bacterial strains isolated and used were the 6 GP bacteria belonging to 3 genera, *S. aureus, Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus saprophyticus* (*S. saprophyticus*), *E. faecalis, S. mutans, S. pyogenes* isolated from clinical samples of patients of the outpatient department of Sum Hospital as well as, from patients admitted into ICUs, wards and cabins of the hospital, using an appropriate medium for a bacterium. These bacterial strains were isolated to pure (axenic) cultures before performing biochemical characterization[60].

2.5. Biochemical identifications and antibiotic sensitivity tests

For pure-cultures of isolated GP cocci, catalase and coagulase tests were performed. Catalase negative colonies were subjected to bile esculin test[13]. All the used 6 bacterial strains were subjected to antibiotic sensitivity test by the disc-diffusion/Kirby-Bauer's


Figure 1. *A. campanulatus*


Figure 2. *C. indica*


Figure 3. *C. tinctorius*


Figure 4. *C. deodara*


Figure 5. *Codiaeum variegaum*


Figure 6. *C. decandrum*


Figure 7. *E. caducifolia*

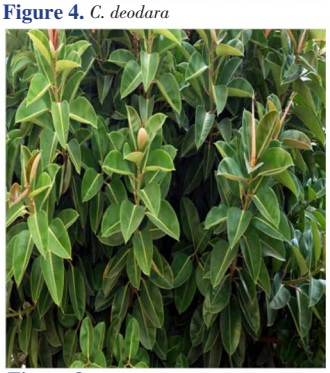
Figure 8. *F. elastica*


Figure 9. *I. coccinea*


Figure 10. *L. aspera*


Figure 11. *Mangifera indica*

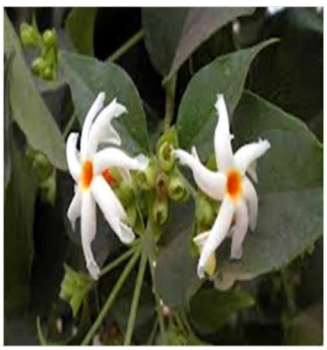
Figure 12. *N. arbor-tristis*

method, with 17 high potency antibiotic-discs (HiMedia), according to CLSI guidelines[61].


Figure 13. *Pisum sativum*


Figure 14. *P. indica*


Figure 15. *P. rubra*


Figure 16. *P. tuberose*


Figure 17. *P. pinnata*


Figure 18. *R. communis*

2.6. Antibacterial activity test by agar-well diffusion method

For monitoring antibacterial activity by the agar-well diffusion method, bacterial lawn was prepared. Wells were punched for 6 mm deep in 30 min old bacterial lawn and each well was based by 50 μL molten Muller-Hilton agar. Further, wells were filled with 100 μL aliquots of 30 mg/mL solvent-extract of a plant (which was diluted from the original stock of plant extract of individual organic solvent, by 10% v/v, DMSO to 30 mg plant-extract/mL, and that of the aqueous plant-extract with water). Plates were incubated at 37 °C for 24 h. Antibacterial activities were evaluated by measuring the diameter values of zones of inhibition. Experiment of each solvent extract was conducted thrice and results of the third repetition are presented. It was confirmed that 10% DMSO had no inhibitory effect on any bacterium. Sterile water was taken as the control for experiments with both cold aqueous phyto-extracts[11,62].

2.7. MIC and MBC values of plant extracts against clinically isolated bacteria

Original stock solutions of plant extracts prepared with water and ethanol (cold extracts) were 50 mg/mL in 10% DMSO solution with distilled water. An aliquot of 80 μL of each dilution of a solvent-extract was released to a well on a 96-welled (12×8) micro-titer plate along with an aliquot of 100 μL nutrient NB (HiMedia, Mumbai), an aliquot of 20 μL bacterial inocula (10^9 CFU/mL) and a 5 μL-aliquot of 0.5% of 2,3,5-triphenyltetrazolium chloride (TTC). After pouring all the above to a well, the micro-plate was incubated at 37 °C for 18 h. A pink colouration in a well

Table 1

Ethnomedicinal information of 30 plants used.

Sl. No.	Plant and family name	Local name, (edibility, E or non-edibility, NE)	Ethnomedicinal uses
1	*A. campanulatus* Decne. Araceae	Olua (E)	The underground stem is edible and is used for curing stomach pain, treating piles and hemorrhages.
2	*Azadirachta indica* A. Juss. Meliaceae	Nimba (E)	Leaf paste made with *Curcuma longa* (turmeric) is used against measles and chicken pox. It is taken orally as well as applied locally.
3	*Calotropis procera* (Ait.) R. Br. Asclepiadaceae	Arakha (NE)	Leaf juice is dropped in to the nostril to treat epilepsy. Root bark paste with opium is applied externally on nostrils to cure nasal sore.
4	*C. indica* L. Var Cannaceae	Kedar (NE)	Leaves are used in the treatment of acute jaundice.
5	*Carica papaya* L. Caricaceae	Amrutabhanda (E)	Fruits of papaya are used to treat high blood pressure, dyspepsia, constipation.
6	*C. tinctorius* L. Asteraceae	Kusuma (E)	Leaves are used for amenorrhoea, dysmenorrhoea and wounds or sores with pain and swelling, and for prevention of atherosclerosis.
7	*C. deodara* (Roxb) Loud Pinaceae	Deodar (NE)	Leaf extract is used for catarrhal conditions of the respiratory tract.
8	*Codiaeum variegaum* (L.) Juss Euphorbiaceae	Croton (NE)	Used to treat amenorrhoea, body aches and eye diseases.
9	*C. decandrum* Roxb. Combretaceae	Atundi (NE)	The seed oil of the plant is used in treating eczema. The raw leaves are eaten to relieve diarrhoea and gastric troubles.
10	*Cucurbita maxima* Duch.ex Lam. Cucurbitaceae	Kakharu (E)	Fruits are used in treating bladder disorders, stomach upsets, wounds, and certain female reproductive complaints.
11	*Diospyros melanoxylon* Roxb. Ebenaceae	Kendu (E)	Leaves are used in urinary tract infection and skin trouble. Bark is used in diarrhea and dyspepsia.
12	*E. caducifolia* Haines Euphorbiaceae	Khira siju (NE)	Plants are used for the detection of diabetes mellitus, erythrocytes surface changes in alcoholics.
13	*Ficus elastica* Roxb. Ex. Hornem Moraceae	Rubber (NE)	Leaves are used in treating constipation, mumps, boils and cardiac weakness. Leaf- paste is applied on wounds and bruises. Bark-paste is administered for jaundice, gonorrhea, ulcers and excessive urination.
14	*Hibiscus rosasinensis* L. Malvaceae	Mandara (NE)	Plant parts are used to cure liver disorders, control high blood pressure. Decoction of leaves, root and fruits are helpful in treatments of arthritis, boils and coughs, and the fruit is used externally in cases of wounds and ulcers.
15	*I. coccinea* L. Rubiaceae	Rangani (NE)	It is used for wound healing, anti-diarrhoeal and anti-inflammatory problems. It is used as haemostatic, antioxidant and anti-ulcerative properties.
16	*L. aspera* Spreng. Lamiaceae	Gayasha (E)	Leaf juice is poured into ear to retrieve ear pain and sores. Leaf juice is taken orally to cure complications due to non-poisonous snakebites. Leaves are rubbed on skin to get relief from itching sensation due to contact of caterpillars.
17	*Mangifera indica* L. Anacardiaceae	Amba (E)	The leaves are used as anti-diabetic, antioxidant, anti-inflammatory, antiviral, hepato-protective, hypoglycemic, anti-allergic and anti-cancerous remedy.
18	*M. koenigii* (L.) Spreng. Rutaceae	Vrusunga (E)	Plant is used for cough, sour eructation, burning sensation, pruritis, skin diseases, anorexia, dyspepsia, colic, flatulence, diarrhoea, dysentery, vomiting, stomatitis and ulcers. It has anti-diabetic and antioxidant effects.
19	*M. sapientum* L. Musaceae	Kadali (E)	Plant sap is used to cure different types of eye infection
20	*N. arbor-tristis* L. Oleaceae	Gangasiuli (NE)	Plant is used to cure inflammation, sciatica, rheumatism, dyspepsia, cough, asthma, constipation, hemorrhoids, baldness, premature graying of hair and pruritus. Useful parts are leaves, flowers and seeds.
21	*Pisum sativum* L. Fabaceae	Matara (E)	Leaf paste is taken orally as contraceptive.
22	*P. indica* L. Plumbaginaceae	Raktachita (NE)	Plants are used in digestive problem, rheumatism and paralysis.
23	*P. rubra* L. Apocynaceae	Katha champa (NE)	Leaves are used in cough, ulcers, skin diseases, inflammations, arthritis and constipation.
24	*P. tuberosa* L. Amaryllidaceae	Tuberose (NE)	The bulbs are dried, powdered and are used as a remedy for gonorrhoea. The bulb is rubbed up with turmeric and butter and applied to neonates to remove small red pimples on their bodies.
25	*P. pinnata* L. Fabaceae	Karanja (NE)	Seed oil is used in coetaneous disease like scabies, herpes and leucoderma.
26	*Psidium guajava* L. Myrtaceae	Pijuli (E)	Leaves are used in wounds, ulcers, rheumatism, and leaves are chewed to relieve toothache. It has been effective in checking vomiting and diarrhoea in cholera patients. It is also applied on skin diseases. A combined decoction of leaves and bark is given to expel the placenta after childbirth.
27	*Punica granatum* L. Punicaceae	Dalimba (E)	It is widely used in treating certain types of cancer including leukemia, breast, prostate and colon cancer, dysentery, diarrhoea, excessive bleeding, expelling intestinal worms and parasites.
28	*Ricinus communis* L. Euphorbiaceae	Castor (E)	It is used to induce labor pain, stimulate lactation, arthritis, contraceptive when applied inside the vagina and on eye lids to soothe irritation.
29	*Syzygium cumini* (L.) Skeels Myrtaceae	Jamukoli (E)	Leaves and bark are used for controlling blood pressure and gingivitis.
30	*Thevetia neriifolia* Pers. Apocynaceae	Kaniar (NE)	Leaves are used as purgative and emetic.

Amorphophalus campanulatus Decne. Araceae (*A. campanulatus*); *Azadirachta indica* A. Juss. Meliaceae (*A. indica*); *Cana indica* L. Var Cannaceae (*C. indica*); *Carthamus tinctorius* L. Asteraceae (*C. tinctorius*); *Cedrus deodara* (Roxb) Loud Pinaceae (*C. deodara*); *Combretum decandrum* Roxb. Combretaceae (*C. decandrum*); *Diospyros melanoxylon* Roxb. Ebenaceae (*D. melanoxylon*); *Euphorbia caducifolia* Haines Euphorbiaceae (*E. caducifolia*); *Ixora coccinea* L. Rubiaceae (*I. coccinea*); *Leueas aspera* Spreng. Lamiaceae (*L. aspera*); *Murraya koenigii* (L.) Spreng. Rutaceae (*M. koenigii*); *Musa sapientum* L. Musaceae (*M. sapientum*); *Nyctanthes arbor-tristis* L. Oleaceae (*N. arbor-tristis*); *Plumbago indica* L. Plumbaginaceae (*P. indica*); *Plumeria rubra* L. Apocynaceae (*P. rubra*); *Polianthes tuberosa* L. Amaryllidaceae (*P. tuberosa*); *Pongamia pinnata* L. Fabaceae (*P. pinnata*); *Ricinus communis* L. Euphorbiaceae (*R. communis*); *Syzygium cumini* (L.) Skeels Myrtaceae (*S. cumini*).

indicated bacterial growth due to TTC and the absence of any colour was taken as the inhibition of bacterial growth. The first well of the micro-titre plate was the control without any plant extract. The MIC value was noted at the well, where no colour was manifested. Further, bacteria from each well of the micro-plate were sub-cultured on a nutrient agar plate; the level of dilution, where no bacterial growth on the agar plate was observed, was noted as the MBC value[11,62]. Experiment of each solvent extract was conducted thrice and results of the third repetition are presented.

3. Results

3.1. Ethnobotany and preliminary phytochemical analyses

Ethnomedicinal information of 30 plants (of which, 15 were edible) with vernacular names from aborigines of Kalahandi District, Odisha along with their modalities, sometimes with several other plants in use were recorded. These plants are in use for diseases, measles, chicken pox, stomach pain, jaundice, diarrhoea, gonorrhoea,

Table 2

Preliminary phytochemical analysis of aqueous and ethanol extracts of 30 plants.

Sl. No.	Flavonoids	Saponins	Phlobatannis	Resins	Sterols	Lipids/ Fats	Steroids	Tannins	Glycosides	Acidic compounds	Terpenoids	Reducing sugar	Phenols	Carbo-hydrates	Anthra-quinones
1	+ (+)	+ (−)	− (−)	− (−)	+ +)	− (−)	− (+)	− (−)	+ (−)	− (+)	+ (+)	− (+)	− (−)	− (−)	− (−)
2	− (−)	+ (−)	− (−)	+ (−)	− (−)	+ (−)	− (+)	+ (+)	+ (+)	− (−)	+ (+)	− (−)	+ (−)	− (−)	− (−)
3	− (+)	+ (+)	− (−)	− (−)	− (−)	+ (−)	− (−)	− (+)	− (−)	− (+)	− (−)	− (+)	− (−)	− (−)	+ (+)
4	− (−)	+ (−)	− (−)	− (+)	− (+)	+ (−)	+ (−)	+ (−)	− (−)	+ (−)	− (−)	− (−)	+ (−)	− (−)	− (−)
5	− (−)	− (−)	+ (−)	+ (−)	− (−)	+ (−)	+ (+)	− (−)	− (−)	+ (+)	+ (+)	− (−)	+ (−)	− (−)	− (−)
6	− (+)	+ (+)	+ (−)	+ (−)	+ (−)	− (−)	+ (+)	+ (+)	+ (+)	− (−)	+ (+)	− (+)	+ (−)	− (+)	+ (+)
7	− (−)	+ (−)	− (−)	− (−)	+ (−)	+ (+)	+ (−)	+ (−)	− (−)	− (−)	− (−)	+ (−)	+ (−)	− (−)	+ (−)
8	− (−)	+ (−)	+ (−)	+ (−)	− (−)	− (−)	+ (−)	+ (+)	− (−)	− (+)	+ (−)	+ (+)	+ (−)	− (−)	+ (−)
9	− (−)	+ (+)	+ (−)	+(+)	− (+)	− (−)	− (+)	+ (−)	+ (+)	− (−)	− (−)	− (−)	+ (−)	− (−)	− (+)
10	− (−)	+ (−)	+ (−)	+ (−)	− (+)	+ (−)	+ (−)	+ (+)	+ (−)	+ (+)	+ (−)	− (−)	+ (−)	− (−)	+ (−)
11	− (+)	+ (−)	+ (−)	+(+)	− (−)	− (−)	+ (−)	+ (−)	− (+)	− (−)	− (−)	+ (+)	− (−)	− (−)	+ (−)
12	− (−)	+ (+)	+ (−)	− (−)	+ (+)	− (−)	− (−)	− (−)	− (−)	− (−)	+ (+)	+ (+)	− (−)	− (−)	+ (−)
13	− (+)	+ (−)	+ (−)	− (+)	− (−)	− (−)	+ (+)	− (+)	− (−)	− (+)	+ (−)	− (−)	− (−)	− (−)	+ (+)
14	− (−)	+ (−)	+ (−)	− (−)	− (+)	+ (−)	+ (−)	+ (+)	+ (−)	− (−)	− (−)	+ (+)	− (−)	− (−)	+ (−)
15	− (−)	+ (−)	− (−)	− (−)	+ (−)	+ (−)	+ (+)	+ (−)	− (−)	− (−)	− (−)	+ (−)	+ (−)	− (−)	+ (+)
16	− (−)	+ (+)	− (−)	− (−)	+ (−)	+ (−)	− (−)	− (−)	− (−)	− (−)	− (−)	+ (−)	+ (−)	− (−)	+ (−)
17	− (+)	+ (−)	+ (−)	− (−)	+ +)	− (−)	− (−)	+ (+)	+ (+)	− (−)	− (+)	− (−)	− (−)	− (−)	+ (−)
18	− (−)	− (−)	+ (−)	+ (−)	+ (−)	− (−)	+ (−)	+ (+)	− (−)	− (+)	− (−)	− (−)	+ (−)	− (−)	+ (+)
19	− (−)	+ (+)	+ (−)	+ (−)	+ (−)	− (−)	+ (+)	− (−)	− (+)	− (−)	− (−)	− (+)	+ (−)	− (−)	+ (−)
20	− (−)	+ (−)	− (−)	+ (+)	− (−)	− (−)	− (−)	+ (−)	+ (−)	− (−)	+ (−)	− (−)	− (−)	− (−)	+ (−)
21	− (+)	+ (−)	− (−)	− (−)	+ (+)	− (+)	+ (+)	+ (+)	+ (−)	+ (+)	+ (+)	− (−)	+ (−)	+ (−)	+ (−)
22	− (−)	+ (−)	− (−)	− (−)	+ (+)	+ (−)	+ (−)	+ (−)	+ (+)	− (−)	− (−)	+ (+)	− (−)	− (−)	+ (−)
23	− (−)	+ (−)	− (−)	− (−)	+ +)	− (−)	+ (+)	+ (+)	+ (−)	− (−)	+ (−)	− (−)	+ (−)	− (+)	+ (−)
24	− (−)	+ (−)	+ (−)	+(−)	+ (−)	− (−)	+ (+)	− (−)	− (+)	− (−)	+ (+)	− (+)	− (−)	− (−)	− (+)
25	− (−)	+ (+)	− (−)	+ (+)	− (+)	− (−)	+ (+)	+ (−)	+ (−)	+ (+)	− (+)	− (−)	− (−)	− (−)	+ (−)
26	− (+)	+ (+)	− (−)	+ (−)	+ (−)	− (+)	+ (+)	+ (+)	+ (+)	− (−)	− (−)	− (−)	+ (−)	− (−)	+ (−)
27	− (−)	+ (−)	− (−)	+(+)	+ (−)	− (−)	+ (+)	− (+)	− (+)	− (−)	− (+)	− (−)	+ (−)	− (−)	+ (−)
28	− (−)	+ (−)	+ (−)	− (−)	+ (−)	− (−)	+ (−)	+ (−)	+ (−)	+ (+)	− (−)	− (+)	− (−)	+ (−)	+ (−)
29	− (−)	+ (+)	− (−)	− (−)	+ (−)	− (−)	+ (+)	− (−)	+ (−)	+ (+)	− (−)	− (−)	− (−)	− (−)	− (−)
30	− (+)	+ (−)	+ (−)	− (+)	+ (−)	− (−)	+ (−)	+ (−)	− (−)	− (−)	− (−)	− (+)	+ (−)	− (−)	+ (−)

No. corresponding to the sequence number in Table 1; Left column refer to aqueous extracts and values in parenthesis ethanol extracts; +: Present; −; Absent.

Table 3

Morphology and culture characters of clinically isolated Gram-positive bacteria along with MTCC strains.

Bacterium	MTCC No.	Agar media	Colony morphology
E. faecalis	439	Nutrient agar	Oval cocci, in a pair arranged at an angle to each other
		Mannitol salt agar	Yellow colonies
		Blood agar	Produces light-black colonies
		MacConkey agar	Tiny deep pink colonies
S. aureus	7443	Nutrient agar	Golden yellow, opaque, circular colonies white butyrous consistency
		Mannitol salt agar	Yellow colonies
		Blood agar	Beta haemolysis
S. epidermidis	NA	Nutrient agar	White, opaque, circular colonies
		Mannitol salt agar	No growth, no haemolysis
S. saprophyticus	NA	Nutrient agar	White, opaque, circular colonies
		Mannitol salt agar	No growth
		Blood agar	No haemolysis
S. mutans	497	6.5% NaCl broth	Growth
		Blood agar	Clear haemolysis around colony
S. pyogenes	1928	Blood agar	Colonies are small circular, semitransparent, low convex discs with an area of clear haemolysis around them
		6.5% NaCl broth	No growth

MTCC: Microbial Type Culture Collections; NA: Not available.

cough, ulcers, skin diseases, inflammations and arthritis, *etc*. (Table 1). Preliminary phytochemical analyses were done for both aqueous and ethanolic extracts of all plants. Ethanolic extracts of most plants had phytochemicals, glycosides, terpenoids, reducing sugars, saponins, tannins, flavonoids and steroids. Aqueous extract of certain plants did not contain flavonoids, but corresponding alcoholic extracts had flavonoids. Obviously, the presence of such phytocompounds in individual extracts cumulatively redounds to

the antibacterial activities of plants. The results of phytochemical analyses of all plants are recorded (Table 2).

3.2. Bacterial identifications

GP bacteria as medium to large, smooth, entire, slightly raised, creamy yellow, with green/β-haemolytic colonies on blood agar, found positive to catalase, coagulase tests were confirmed as *S. aureus*. Catalase negative GP colonies showing β-haemolysis (complete haemolysis of erythrocytes) on blood agar and simultaneously sensitive to bacitracin were identified as Group A streptococci or *S. pyogenes*. Further, bile-esculin producing colonies, negative to catalase test were taken as *E. faecalis*, which produced greyish, round, small colonies with alpha-haemolytic zones on blood agar. Similarly, the rest three GP bacteria were identified (Tables 3 and 4, Figures 19-24).

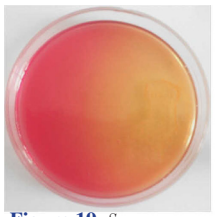

Figure 19. *S. aureus*

Figure 20. *E. faecalis*

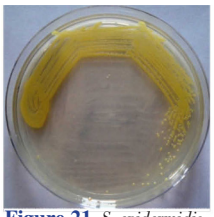

Figure 21. *S. epidermidis*

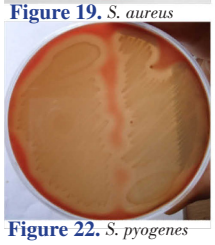

Figure 22. *S. pyogenes*

Figure 23. *S. mutans*

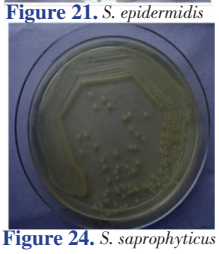

Figure 24. *S. saprophyticus*

Table 4

Summary of results of biochemical tests of MDR GP bacteria.

Bacteria	Catalase	Coagulase	Bile esculin	Novobiocin test
E. faecalis	-ve	-ve	+ve	Nd
S. aureus	+ve	+ve	Nd	Sensitive
S. epidermidis	+ve	-ve	Nd	Sensitive
S. saprophyticus	+ve	-ve	Nd	Resistant
S. mutans	-ve	-ve	-ve	Nd
S. pyogenes	-ve	-ve	-ve	Nd

-ve: Negative; +ve: Positive; Nd: not done.

3.3. Antibiograms of bacteria

Among the 6 GP bacteria, *E. faecalis* was found resistant to 10 antibiotics out of 17 antibiotics whereas, it was found sensitive to 6 antibiotics including ampicillin, oxacillin, teicoplanin, vancomycin, azithromycin and linezolid and moderately sensitive to ofloxacin. Likewise *S. aureus* was found resistant to 8 antibiotics out of 17 prescribed antibiotics whereas, it was sensitive to 8 antibiotics and moderately sensitive to one, azithromycin. Again, *S. pyogenes* was found resistant to all the 17 antibiotics used. Similarly, the antibiotic sensitivity pattern of the rest three GP bacteria were recorded (Table 5). Percent values of each of 6 GP pathogens resistant to individual antibiotics of 9 antibiotic groups were recorded (Table 6). *E. faecalis* had the highest 73% resistance value to oxacillin, followed by 72% to ceftriaxone, 69% to chloramphenicol and the least value 23% to vancomycin. Likewise, *S. aureus* had the highest 87% resistance value to oxacillin, followed by 85% to ceftriaxone, 75% to amikacin and the least resistance value 27% to vancomycin. Similarly, the resistance percent values of the rest other GP bacteria with all antibiotics used were recorded (Table 6).

Table 5

Antibiotic susceptibility results of MDR GP bacteria.

Bacteria	Aminoglycosides		β-lactams				Cephalosporins		Fluoroquinolone	Glycopeptides		Macrolides		Lincosamide	Sulfonamide	Stand alone	
	Ac	Ge	Ak	Am	Ox	P	Ctr	Cf	Of	Tei	Va	E	Az	Cd	Cot	Ch	Lz
E. faecalis	R	R	R	S	S	R	R	R	MS	S	S	R	S	R	R	R	S
S. aureus	R	R	S	S	R	R	S	R	S	S	S	R	MS	S	R	R	S
S. saprophyticus	R	R	R	R	R	R	R	R	R	MS	R	R	MS	R	R	R	S
S. epidermidis	R	R	R	MS	R	R	R	R	R	S	S	R	R	R	S	S	S
S. mutans	R	R	MS	R	R	R	R	R	MS	R	R	R	R	R	R	R	S
S. pyogenes	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Ac: Amikacin (30 μg/disc); Ak: Amoxyclav (30 μg/disc); Am: Ampicillin (10 μg/disc); Az: Azithromycin (15 μg/disc); Cd: Clindamycin (2 μg/disc); Cf: Cefpodoxime (10 μg/disc); Ch: Chloramphenicol (30 μg/disc); Cot: Co-trimoxazole (25 μg/disc); Ctr: Ceftriaxone (30 μg/disc); E: Erythromycin (15 μg/disc); Ge: Gentamicin (10 μg/disc); Lz: Linezolid (30 μg/disc); Of: Ofloxacin (5 μg/disc); Ox: Oxacillin (1 μg/disc); P: Penicillin (10 μg/disc); Tei: Teicoplanin (30 μg/disc); Va: Vancomycin (30 μg/disc).

Table 6

Percentage of antibiotic resistance pattern of MDR GP bacteria.

Bacteria	Aminoglycosides		β-lactams				Cephalosporin		Fluoroquinolone	Glycopeptides		Macrolides		Lincosamide	Sulfonamide	Stand alone	
	Ac	Ge	Ak	Am	Ox	P	Ctr	Cf	Of	Tei	Va	E	Az	Cd	Cot	Ch	Lz
E. faecalis	67	38	42	37	73	62	72	52	46	40	23	45	54	25	43	69	32
S. aureus	75	46	43	38	87	59	85	68	53	48	27	43	62	39	48	59	53
S. epidermidis	62	32	22	27	17	48	68	28	23	16	3	27	45	29	37	48	43
S. saprophyticus	59	43	26	25	24	36	72	29	33	32	7	19	25	39	34	29	39
S. mutans	69	52	58	42	38	37	68	32	48	38	17	23	42	35	52	38	33
S. pyogenes	58	39	32	27	68	67	58	32	38	57	8	13	25	37	54	35	27

Ac: Amikacin (30 μg/disc); Ak: Amoxyclav (30 μg/disc); Am: Ampicillin (10 μg/disc); Az: Azithromycin (15 μg/disc); Cd: Clindamycin (2 μg/disc); Cf: Cefpodoxime (10 μg/disc); Ch: Chloramphenicol (30 μg/disc); Cot: Co-trimoxazole (25 μg/disc); Ctr: Ceftriaxone (30 μg/disc); E: Erythromycin (15 μg/disc); Ge: Gentamicin (10 μg/disc); Lz: Linezolid (30 μg/disc); Of: Ofloxacin (5 μg/disc); Ox: Oxacillin (1 μg/disc); P: Penicillin (10 μg/disc); Tei: Teicoplanin (30 μg/disc); Va: Vancomycin (30 μg/disc).

3.4. Antibacterial activity of plants

Antibacterial activity of aqueous and ethanol extracts of 30 plants were tested by the agar-well diffusion method. A MDR *E. faecalis* strain was highly susceptible to the aqueous extracts of plants *D. melanoxylon*, *N. arbor-tristis*, *P. indica*, *P. rubra* and *P. pinnata* whereas, moderately susceptible to *A. campanulatus*, *A. indica*, *C. indica*, *C. decandrum*, *M. koenigii* and *M. sapientum*. Aqueous extracts of the rest 19 plants were totally effective to control the MDR *E. faecalis* strain *in vitro*. Similarly, ethanolic extracts of *C. indica*, *C. tinctorius*, *D. melanoxylon*, *I. coccinea*, *M. koenigii*, *P. rubra*, *P. pinnata* and *S. cumini* were highly effective in controlling the *E. faecalis* MDR strain whereas, ethanolic extracts of the rest 22 plants were moderately effective. Likewise, the effectivity both aqueous and ethanolic extracts of the 30 plants against the rest MDR 5 GP bacteria were recorded (Table 7).

Table 7

Antibacterial activity of aqueous and ethanol extracts of selected plants by the agar well diffusion method against MDR GP bacteria.

Sl. No.	*E. faecalis*	*S. aureus*	*S. epidermidis*	*S. saprophyticus*	*S. mutans*	*S. pyogenes*
1	15 (13)	17 (14)	22 (19)	22 (20)	17 (20)	19 (23)
2	17 (19)	16 (19)	18 (23)	15 (26)	15 (19)	18 (19)
3	– (16)	– (–)	15 (19)	– (16)	15 (18)	18 (20)
4	19 (23)	20 (19)	24 (20)	23 (22)	19 (18)	19 (21)
5	– (17)	14 (22)	16 (19)	17 (26)	15 (20)	15 (18)
6	– (21)	– (18)	17 (21)	– (22)	19 (18)	18 (20)
7	– (19)	– (14)	– (18)	– (12)	– (15)	15 (19)
8	– (15)	– (–)	13 (17)	18 (22)	– (17)	– (15)
9	15 (19)	14 (18)	18 (20)	15 (27)	17 (15)	12 (17)
10	– (16)	– (–)	– (16)	– (–)	– (19)	– (16)
11	21 (26)	19 (21)	19 (26)	22 (25)	20 (24)	23 (27)
12	– (15)	– (19)	18 (20)	14 (20)	– (–)	– (14)
13	– (16)	12 (19)	12 (16)	17 (18)	– (15)	– (17)
14	– (15)	– (–)	– (18)	– (15)	15 (18)	– (–)
15	24 (25)	23 (25)	24 (25)	21 (24)	23 (24)	25 (26)
16	– (15)	– (19)	– (17)	15 (22)	15 (20)	15 (19)
17	– (18)	– (15)	14 (19)	– (22)	12 (22)	15 (20)
18	16 (20)	12 (21)	15 (19)	– (24)	19 (21)	20 (25)
19	16 (19)	15 (22)	14 (20)	– (22)	15 (20)	19 (22)
20	22 (24)	25 (26)	24 (27)	25 (27)	24 (26)	21 (23)
21	– (–)	– (14)	– (18)	– (20)	15 (17)	17 (20)
22	26 (19)	24 (17)	36 (18)	33 (22)	18 (22)	15 (20)
23	24 (27)	23 (25)	24 (27)	22 (24)	21 (24)	25 (26)
24	– (19)	15 (20)	18 (22)	16 (27)	– (20)	– (17)
25	24 (26)	23 (26)	21 (25)	20 (22)	21 (25)	22 (24)
26	– (18)	– (14)	– (–)	– (–)	– (17)	– (18)
27	– (17)	– (18)	– (20)	– (22)	– (17)	– (17)
28	– (18)	14 (18)	14 (17)	– (20)	– (15)	– (17)
29	25 (26)	22 (26)	20 (24)	21 (22)	21 (25)	24 (27)
30	– (14)	– (17)	12 (21)	15 (22)	16 (23)	16 (19)

Numbers 1 to 30 are serial numbers of plants given in Table 1; Left column of values are measurements of zones of inhibition due to aqueous extracts and values in parenthesis are due to ethanol extracts.

Plants with most conspicuous antibacterial properties for each bacterium are presented in Table 8. Two independent student's *t*-tests were conducted, one for number of bacteria controlled by each of water or ethanolic extract, while the second test was with number of effective plants against a bacterium, with same '30 plants 6 GP MDR bacteria' combination. The first test, was conducted for each MDR bacterium (Table 8), the df=30-1=29, the calculated t-value=4.18 was greater than the tabulated t-value=3.66, at P=0.001 level, rejecting the null hypothesis that 'both extracts were equally effective', at P=0.001 level. In other words, ethanolic extract was more effective than the corresponding aqueous extract of each plant in controlling 6 MDR GP bacteria. Similarly, the second t-test was conducted between the numbers of effective aqueous or ethanolic extracts of 30 plants against individual clinically isolated MDR bacteria (Table 9). With df=6-1=5, the calculated t= 6.9 was greater than the tabulated t=6.87 at P=0.001 level, the difference between effective aqueous and ethanol extract was highly significant at P=0.001 level; thus, the statement that 'ethanolic extracts were effective than aqueous extracts' was true in 99.99% cases with GP bacteria.

Table 8

Number of plant of leaf of aqueous extract and ethanol extract sensitive to MDR GP bacteria.

Sl. No.	Gram-positive bacteria	
	Aqueous extract	Ethanol extract
1	6	6
2	6	6
3	3	5
4	6	6
5	5	6
6	3	3
7	1	6
8	2	5
9	6	6
10	0	4
11	6	6
12	2	5
13	3	6
14	1	4
15	6	6
16	3	6
17	3	6
18	5	6
19	5	6
20	6	6
21	2	5
22	6	6
23	6	6
24	3	6
25	6	6
26	0	4
27	0	6
28	2	6
29	6	6
30	4	4
Mean±SD	3.77±2.14	5.50±0.86

Numbers 1 to 30 are serial numbers of plants given in Table 1; The student's *t*-test was conducted (see text).

Table 9

Number of MDR GP bacteria sensitive to leaf of aqueous extract and ethanol extract.

Bacteria	Total number of aqueous extract	Total number of ethanol extract
E. faecalis	1,2,4,9,11,15,18,19,20,22,23,25,29 (13)	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,22,23,24,25,26,2728,29,30 (28)
S. aureus	1,2,4,5,9,11,13,15,18,19,20,22,23,24,25, 28,29 (17)	1,2,4,5,6,7,9,10,11,12,13,15,16,17,18,19, 20,21,22,23,24,25,26,27,28,29,30 (27)
S. epidermidis	1,2,3,4,5,6,8,9,,11,12,13,15,17,18,19,20, 22,23,24,25,28,29,30 (23)	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,27,28,29,30 (29)
S. saprophyticus	1,2,4,5,8,9,11,12,13,15,16,20,22,23,24, 25,29,30 (18)	1,2,3,4,5,6,7,8,9,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,27,28,29,30 (28)
S. mutans	1,2,3,4,5,6,9,11,14,15,16,17,18,19,20, 21,22,23,25,29,30 (21)	1,2,3,4,5,6,7,8,9,10,11,13,14,15,16,17,18,19, 20,21,22,23,24,25,26,27,28,29,30 (29)
S. pyogenes	1,2,3,4,5,6,7,9,11,15,16,17,18,19,20,21, 22,23,25,29,30 (21)	1,2,3,4,5,6,7,8,9,10,11,12,13,15,16,17,18,19, 20,21,22,23,24,25,26,27,28,29,30 (29)
Mean±SD	18.83±3.60	28.33±0.82

The student's *t*-test was conducted (see text).

Table 10

MIC of 30 plants against MDR GP bacteria (mg/mL).

Sl. No.	*E. faecalis*	*S. aureus*	*S. epidermidis*	*S. saprophyticus*	*S. mutans*	*S. pyogenes*
1	1.56 (3.12)	1.56 (3.12)	0.78 (1.56)	0.78 (0.78)	1.56 (0.78)	1.56 (0.78)
2	1.56 (1.56)	1.56 (1.56)	1.56 (0.78)	1.56 (26.00)	1.56 (1.56)	1.56 (1.56)
3	– (1.56)	– (–)	1.56 (1.56)	– (1.56)	1.56 (1.56)	1.56 (0.78)
4	1.56 (0.78)	0.78 (1.56)	0.78 (0.78)	0.78 (0.78)	1.56 (1.56)	1.56 (0.78)
5	– (1.56)	3.12 (0.78)	1.56 (1.56)	1.56 (26.00)	1.56 (0.78)	1.56 (1.56)
6	– (0.78	– (1.56)	1.56 (0.78)	– (0.78)	1.56 (1.56)	1.56 (0.78)
7	– (1.56)	– (3.12)	– (1.56)	– (6.25)	– (1.56)	1.56 (1.56)
8	– (1.56)	– (–)	3.12 (1.56)	1.56 (0.78)	– (1.56)	– (1.56)
9	1.56 (1.56)	3.12 (1.56)	1.56 (0.78)	1.56 (27.00)	1.56 (1.56)	6.25 (1.56)
10	– (1.56)	– (–)	– (1.56)	– (–)	– (1.56)	– (1.56)
11	0.78 (26.00)	1.56 (0.78)	1.56 (26.00)	0.78 (0.39)	0.78 (0.78)	0.78 (27.00)
12	– (1.56)	– (1.56)	1.56 (0.78)	3.12 (0.78)	– (–)	– (3.12)
13	– (1.56)	6.25 (1.56)	6.25 (1.56)	1.56 (1.56)	– (1.56)	– (1.56)
14	– (1.56)	– (–)	– (1.56)	– (1.56)	1.56 (1.56)	– (–)
15	0.78 (0.39)	0.78 (0.39)	0.78 (0.39)	0.78 (0.78)	0.78 (0.78)	0.39 (26.00)
16	– (1.56)	1.56 (0.78)	– (1.56)	– (1.56)	1.56 (1.56)	1.56 (0.78)
17	3.12 (1.56)	– (0.78)	– (1.56)	– (1.56)	1.56 (0.78)	6.039 (0.78)
18	1.56 (1.56)	– (0.78)	0.39 (0.78)	1.56 (0.78)	0.78 (0.39)	1.56 (0.78)
19	3.12 (0.78)	– (0.78)	1.56 (0.78)	1.56 (1.56)	1.56 (0.78)	1.56 (0.78)
20	0.78 (0.39)	0.39 (0.39)	0.39 (0.39)	0.78 (0.78)	0.78 (0.78)	0.78 (0.39)
21	– (1.56)	– (0.78)	– (3.12)	– (–)	1.56 (0.78)	1.56 (1.56)
22	36.00 (1.56)	33.00 (0.78)	0.78 (1.56)	0.39 (1.56)	1.56 (0.78)	1.56 (0.78)
23	0.78 (0.39)	0.78 (0.78)	0.78 (0.39)	0.78 (0.39)	0.39 (0.39)	0.78 (0.78)
24	1.56 (0.78)	1.56 (0.39)	1.56 (0.78)	– (1.56)	– (1.56)	– (0.78)
25	0.78 (0.39)	0.78 (0.78)	0.78 (0.39)	0.78 (0.39)	0.78 (0.78)	0.78 (0.39)
26	– (–)	– (–)	– (3.12)	– (1.56)	– (1.56)	– (1.56)
27	– (0.78)	– (0.78)	– (1.56)	– (1.56)	– (1.56)	– (1.56)
28	3.12 (1.56)	– (0.78)	3.60 (0.39)	– (1.56)	– (1.56)	– (1.56)
29	0.78 (0.78)	0.78 (0.78)	0.78 (0.39)	0.39 (0.39)	0.78 (0.39)	0.78 (0.39)
30	0.39 (0.78)	1.56 (0.78)	– (1.56)	– (3.12)	1.56 (1.56)	1.56 (0.78)

Numbers 1 to 30 are serial numbers of plants given in Table 1; Left column of values are MIC of aqueous extracts and values in parenthesis are due to ethanol extracts.

MIC values of both ethanolic and aqueous extracts of 30 plants against 6 GP bacteria were determined. It was found that with *S. aureus*, the minimum MIC value was 0.39 mg/mL by the aqueous extract of *N. arbor–tristis*, while it was 0.39 mg/mL by ethanolic extracts of plants, *I. coccinea*, *N. arbor–tristis* and *P. tuberosa*. With *E. faecalis*, the minimum MIC value was 0.78 mg/mL by aqueous extracts of *D. melanoxylon*, *I. coccinea*, *N. arbor–tristis* and *P. pinnata*; while it was 0.39 mg/mL by ethanolic extracts of plants, *I. coccinea*, *N. arbor–tristis*, *P. rubra* and *P. pinnata*. With *S. epidermidis*, the minimum MIC value was 0.39 mg/mL by the aqueous extract of *N. arbor–tristis*; while it was 0.39 mg/mL by ethanolic extracts of plants, *N. arbor–tristis*, *P. rubra*, *P. pinnata*, *R. communis*, *S. cumini*, and *I. coccinea*. With *S. saprophyticus*, the minimum MIC value was 0.39 mg/mL by aqueous extracts of *P. indica*, *S. cumini*; while it was 0.39 mg/mL by ethanolic extracts of plants, *D. melanoxylon*, *P. rubra*, *P. pinnata* and *S. cumini*. With *S. mutans*, the minimum MIC value was 0.39 mg/mL by the aqueous extract of *P. rubra*, while it was 0.39 mg/mL by ethanolic extracts of plants, *M. koenigi*, *P. rubra* and *S. cumini*. With *S. pyogenes*, the minimum MIC value was 0.39 mg/mL by the aqueous extract of *I. coccinea*, while it was 0.39 mg/mL by ethanolic extracts of plants *N. arbor–tristis*, *P. pinnata* and *S. cumini* (Table 10).

Table 11

MBC of 30 plants against MDR GP bacteria (mg/mL).

Sl. No.	*E. faecalis*	*S. aureus*	*S. epidermidis*	*S. saprophyticus*	*S. mutans*	*S. pyogenes*
1	6.25 (25.00)	25.50 (25.00)	3.25 (6.25)	3.25 (6.25)	25.5 (6.25)	6.25 (3.25)
2	25.50 (6.25)	6.25 (6.25)	6.25 (3.25)	6.25 (0.78)	6.25 (6.25)	6.25 (6.25)
3	– (6.25)	– (–)	6.25 (6.25)	– (6.25)	6.25 (6.25)	6.25 (6.25)
4	6.25 (3.25)	6.25 (6.25)	3.25 (6.25)	3.25 (3.25)	6.25 (6.25)	6.25 (3.25)
5	– (25.50)	25.00 (3.25)	6.25 (6.25)	25.5 (0.78)	6.25 (6.25)	6.25 (6.25)
6	– (3.25)	– (6.25)	25.50 (3.25)	– (3.25)	6.25 (6.25)	6.25 (6.25)
7	– (6.25)	– (25.00)	– (6.25)	– (25.00)	– (6.25)	6.25 (6.25)
8	– (6.25)	– (–)	25.00 (25.50)	6.25 (3.25)	– (25.50)	– (6.25)
9	6.25 (6.25)	25.00 (6.25)	6.25 (6.25)	6.25 (0.78)	25.5 (6.25)	25.00 (25.50)
10	– (6.25)	– (–)	– (6.25)	– (–)	– (6.25)	– (6.25)
11	3.25 (0.78)	6.25 (3.25)	6.25 (0.78)	3.25 (1.56)	6.25 (3.25)	3.25 (0.78)
12	– (6.25)	– (6.25)	6.25 (6.25)	25.00 (6.25)	– (–)	– (25.00)
13	– (6.25)	25.00 (6.25)	25.00 (6.25)	25.50 (6.25)	– (6.25)	– (25.50)
14	– (6.25)	– (–)	– (6.25)	– (6.25)	6.25 (6.25)	– (–)
15	3.25 (1.56)	3.25 (1.56)	3.25 (1.56)	3.25 (3.25)	3.25 (3.25)	1.56 (0.78)
16	– (6.25)	– (3.25)	– (3.25)	6.25 (1.56)	6.25 (3.25)	6.25 (3.25)
17	– (3.25)	– (6.25)	25.50 (3.25)	– (1.56)	6.25 (3.25)	25.00 (1.56)
18	6.25 (3.25)	25.00 (1.56)	6.25 (3.25)	– (1.56)	3.25 (1.56)	3.25 (1.56)
19	6.25 (3.25)	6.25 (1.56)	25.50 (3.25)	– (1.56)	3.25 (1.56)	6.25 (3.25)
20	1.56 (1.56)	1.56 (0.78)	1.56 (0.78)	1.56 (0.78)	1.56 (1.56)	1.56 (0.78)
21	– (–)	– (25.50)	– (3.25)	– (3.25)	3.25 (3.25)	6.25 (3.25)
22	0.78 (3.25)	1.56 (3.25)	36.00 (3.25)	33.00 (1.56)	6.25 (3.25)	3.25 (1.56)
23	1.56 (0.78)	1.56 (1.56)	1.56 (0.78)	1.56 (1.56)	1.56 (0.78)	1.56 (1.56)
24	– (3.25)	6.25 (3.25)	3.25 (1.56)	6.25 (0.78)	– (3.25)	– (3.25)
25	1.56 (0.78)	1.56 (0.78)	1.56 (1.56)	3.25 (1.56)	1.56 (1.56)	1.56 (1.56)
26	– (3.25)	– (25.50)	– (–)	– (–)	– (3.25)	– (3.25)
27	– (3.25)	– (3.25)	– (3.25)	– (1.56)	– (3.25)	– (3.25)
28	– (3.25)	25.50 (3.25)	25.50 (3.25)	– (3.25)	– (3.25)	– (6.25)
29	1.56 (0.78)	1.56 (0.78)	3.25 (1.56)	1.56 (1.56)	1.56 (0.78)	1.56 (1.56)
30	– (25.5)	– (3.25)	25.00 (1.56)	6.25 (1.56)	6.25 (3.25)	6.25 (1.56)

Numbers 1 to 30 are serial numbers of plants given in Table 1. Left column of values are MBC of aqueous extracts and values in parenthesis are due to ethanol extracts.

MBC values of both ethanolic and aqueous extracts of 30 plants against all the 6 GP pathogenic bacteria were determined. It was found that with *S. aureus*, the minimum MBC value was 1.56 mg/mL by the aqueous extracts of *S. cumini*, *P. pinnata*, *P. rubra*, *P. indica*, *N. arbor-tristis*, while it was 0.78 mg/mL by ethanolic extracts of plants, *N. arbor-tristis*, *P. pinnata*, and *S. cumini*. With *E. faecalis*, the minimum MBC value was 0.78 mg/mL by the aqueous extract of *P. indica*, while it was 0.78 mg/mL by ethanolic extracts of plants, *S. cumini*, *P. pinnata*, *P. rubra* and *D. melanoxylon*. With *S. epidermidis*, the minimum MBC value was 1.56 mg/mL by aqueous extracts of *P. pinnata*, *P. rubra*, *N. arbor-tristis*, while it was 0.78 mg/mL by ethanolic extracts of plants, *D. melanoxylon*, *N. arbor-tristis*, and *P. rubra*. With *S. saprophyticus*, the minimum MBC value was 1.56 mg/mL by aqueous extracts of *N. arbor-tristis* and *S. cumini*, while it was 0.78 mg/mL by ethanolic extracts of plants, *A. indica*, *C. papaya*, *C. decandrum*, *N. arbor-tristis* and *P. tuberosa*. With *S. mutans*, the minimum MBC value was 1.6 mg/mL by aqueous extracts of *N. arbor-tristis*, *P. rubra*, *P. pinnata*, *S. cumini*, while it was 0.78 mg/mL by ethanolic extracts of plants, *P. rubra*, and *S. cumini*. With *S. pyogenes*, the minimum MBC value was 1.536 mg/mL by aqueous extracts of *I. coccinea*, *N. arbor-tristis*, *P. rubra*, *P. pinnata* and *S. cumini*, while it was 0.78 mg/mL by ethanolic extracts of plants, *D. melanoxylon*, *I. coccinea* and *N. arbor-tristis* (Table 11).

4. Discussion

MDR pathogenic bacterial strains shiver down a hospital's spine by spreading nosocomial infections. Indeed, the available armamentaria with antimicrobial stewardship programme against MDR pathogenic bacteria are slowly narrowed/diminished[1], since the slower rate of addition of newer antibiotics by apothecary.

The poverty-stricken and marginalized section in India consisting of aborigine tribes living in hilly areas continue to depend on plant/herbal products from the local forest-patch for all basic needs including the health care. Plants, for the health-care needs of the numerically important aborigine tribe, Kandha tribe, were described from Odisha state[19,63]. Located at the eastern range of mountains in the state, with a 40% aborigine population, Kalahandi district is richer in vegetations in comparison to other hilly patches of the state. These people maintain their ethnomedicinal knowledge orally in a surreptitious way down the generations, but young adults of the society migrate from their base for livelihood to urban areas; eventually, they lose the chance of knowing medicinal plants. Moreover, regular episodes of summer forest fire in the district as well as in several other places of Indian forest patches lead to inexorable and insurmountable loss of vegetations, entailing environmental degradations at all angles of fire catching zones[19,64]. What was worse, unsustainable harnessing of prodigious forest products including plant parts for the medicinal plant trade and timber causes a blasting diminution of phytodiversity and concomitant shape-shifting of the total forest that becomes unsuitable to contain the usual flora and fauna. Eventually, the creation of forest patches becomes so common that induces survey work of medicinal plants at different areas. Furthermore, India with tropical and sub-tropical forest areas is a home to 550 million plants approximately that serve as the source of traditional medicine (TM), derived from the clandestine ethnic information and Ayurveda[65]. Plants involved in TM have been in use in several ways and they are popular with a'la carte menu-like concoctions and specific modalities, idiosyncratic to 'medicines and diseases', which have facilitated the modern drug development and the use of finished herbal medicines as different formulations, in the 'Herbal-medicine-trade'[66]. TM as a field remains as the major accessible and affordable method of treatment for disease of marginalized people and aborigines, as the old social paradigm. Moreover, TM has been in use in several developed western countries, as an important mode of CAM[67-69]. For example, 48% of population in Australia, 70% in Canada, 42% in USA, 38% in Belgium, and 75% in France use CAM, as it is known[3]; nevertheless, the most popular herbal medicines of the trade do not have institutional/scientific/clinical/pharmaceutical validation for the direct use as drugs in the mainstream medicine. Such crude phyto-drugs are available in market shelves everywhere and elite person love to lean to them, for well-being or health boosting. Many a crude concoctions of phyto-drugs are preventives, but their curative roles are mostly not established.

Moreover, most phyto-drugs cannot be ensconced for some ailments directly as morphine or quinine has been, but for host-toxicity testing. However, do we have the time for such studies with a myriad of phytochemicals, systematically and scientifically for the control of the avalanche of MDR pathogenic bacteria, spatting in both community and nosocomial settings in almost all countries? Logistically, the host-toxicity testing of crude phyto-drugs with mammals as antimicrobials needs be verified, but those do not serve the exact mirror of toxicity in man. Additionally, several attempts on monitoring of the synergistic effects of crude plant extracts with an obsolete antibiotic for the control of MDR bacteria *in vitro* have been undertaken[59]. In fact, a cohort of MDR entero- and uro-pathogenic bacteria had been controlled *in vitro* by crude phyto-extracts from plants[8,11,70].

The exquisite stress of phyto-drugs as the natural mixture of different classes of compounds in a crude plant-extract is an unbreachable barrier; consequently, MDR bacteria however well-studded with the armamentaria of multidrug resistance, could not win over the crude extract of any plant generally, and specifically if extracts were from non-edible/poisonous plants. In this perspective, the non-committal attitude on crude phyto-drugs for the use as antimicrobials, but seeking pure phytochemicals only for the purpose, would be tantamount to the love for academic/scientific study only, but it would not be an attempt for an immediate practical solution in the crusade against the fast evolving MDR pathogens. However, the search for pure chemicals from phyto-drugs, as drugs should continue for the ultimate goal of holistic control of diseases. As has been seen from myriads of reports on antimicrobial activities of medicinal

plants against drug sensitive/standard bacterial strains of culture collection centers where crude extracts invariably control bacterial strains *in vitro*. Thus, undermining crude phyto-extracts as drugs would decrease the credibility of medicinal plants and induce frenzy attitude against the drug targeting endeavour.

Plants remain the most tangible source of antimicrobials, as ethnomedicinal and folklore reports record age-old practices of the control of infectious diseases by aborigine/ethnic people all over, with herbal products. For example, antibacterial activities of the weed, *Argemone mexicana* were recorded against MDR *P. aeruginosa*, wherein leaf-extracts of the weed with ethanol, methanol and acetone had prominent antipseudomonad activity[62]. Moreover, most ferns are non-edible plants, causing aversions to grazing animals; in a study, it was seen that the creeping fern, *Lygodium flexuosum* had a good control capacity over five MDR strains of GNs, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus* and *Pseudomonas*[71]. In a study exclusively with ten MDR enteropathogens, ethanolic extracts of *Aegle marmelos*, *Holarrhena antidysenterica*, *Cassia fistula*, *Terminalia arjuna* and *Salvadora persica* registered remarkable *in vitro* antibacterial activities[72]. Furthermore, MDR strains of six uropathogens were checked for their susceptibility to 25 plants where, *Aegle marmelos*, *Holarrhena antidysenterica*, and additionally, *Withania somnifera* registered equally remarkable *in vitro* antibacterial activities[72]. MDR *A. baumannii* and *P. aeruginosa* strains were well controlled by the methanolic extract of the weed, *Lantana camara*, in a recent study[11]. Thus, crude phyto-extracts were seen amply controlling MDR strains of diverse pathogens, as conjectured from this and previous studies.

Plants with most conspicuous antibacterial properties in controlling MDR strains of GN bacteria were aqueous and ethanolic extracts of plants, *C. tinctorius*, *Cucurbita maxima*, *M. koenigii*, *L. aspera*, *P. indica* and *Psidium guajava*. Similarly, aqueous and ethanolic extracts of plants *I. coccinea*, *N. arbor-tristis*, *P. rubra*, *P. pinnata* and *S. cumini* were the most effective against the isolated GP bacteria. Extracts of *C. deodara*, *M. sapientum* and *E. caducifolia* had the least antibacterial activity. In general, with the ethanolic extracts, antibacterial activities were recorded better than with the corresponding aqueous extracts. It is dare to think of crude phyto-drugs to be used as CAM during empiric therapy in the treatment of an infectious disease from MDR bacteria. And crude extracts as CAM, if scaled up, could trigger business tycoons as antimicrobials, when the astonishing popularity of whole-plant concoctions in all nations is considered, holistically.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Due to prevalence of MDR pathogenic bacteria in hospital and community settings, there is an urgent need of certain novel control antibacterials. Crude phyto-extracts for the control would be a prudent solution as CAM.

Research frontiers

Multidrug resistant bacteria must be controlled by novel agent(s) along with main-stream medicine to avoid problems in empiric therapy. A failure in empiric therapy before culture report of a clinical sample could lead to infections at innards.

Related reports

Shil *et al.* in 2014 (*J Ethnopharmacol* 2014; 152: 135-141.) have published a research which is related to indigenous knowledge of medicinal plants used by the Reang tribe of Tripura State of India.

Innovations & breakthroughs

When crude phyto-extracts for the control would be used as CAM along with main-stream medicine, many a un-to-ward morbidities and hospital related costs could be avoided. Of course, for non-edible plants host toxicity testing would be mandatory for formal promotion as drugs.

Applications

This work embodies an in-depth study with all available/isolated Gram-positive pathogenic bacteria generally in a hospital set-up. And control agents were 30 common and non-common plants; some of these plants could be further taken up by pharmacy in designing concoctions of phyto-drugs as non-microbial antimicrobials.

Peer review

Methodology is universally accepted. Data were well presented. Since plant extracts had an array of compounds, the control of MDR bacteria was easy. Thus, plant extracts could be used as CAM. These 30 plants are used by Odishan ethnic tribes as traditional medicine.

References

[1] Khan AU, Raffaele Z. *Multidrug resistance: a global concern*. Sharjah: Bentham Science; 2011.
[2] Sen S, Chakraborty R, De B. Challenges and opportunities in the advancement of herbal medicine: India's position and role in a global context. *J Herb Med* 2011; **1**: 67-75.
[3] WHO. WHO traditional medicine strategy 2011. WHO/EMP/MIE/2011.2.3. Geneva: World Health Organization; 2011, p. 1-14.

[Online] Available from: http://apps.who.int/medicinedocs/documents [Accessed on 15th Jan, 2013]
[4] Rates SM. Plants as source of drugs. *Toxicon* 2001; **39**: 603-613.
[5] Dubey D, Rath S, Sahu MC, Nayak N, Debata NK, Padhy RN. Status of multidrug resistance in tubercle bacillus and phytochemicals for the control. *J Pub Health* 2013; **21**: 115-119.
[6] Sahu MC, Dubey D, Rath S, Debata NK, Padhy RN. Multidrug resistance of *Pseudomonas aeruginosa* as known from surveillance of nosocomial and community infections in an Indian teaching hospital. *J Pub Health* 2012; **20**: 413-423.
[7] Dubey D, Rath S, Sahu MC, Debata NK, Padhy RN, Olajubu FA. A report on infection dynamics of inducible clindamycin resistance of *Staphylococcus aureus* isolated from a teaching hospital in India. *Asian Pac J Trop Biomed* 2013; **3**: 148-153.
[8] Rath S, Padhy RN. Surveillance of multidrug resistance of 10 enteropathogens in a teaching hospital and *in vitro* efficacy of 25 ethnomedicinal plants used by an Indian aborigine. *Asia Pac J Trop Dis* 2012; **2**(Suppl 1): S336-S346.
[9] Velvizhi G, Sucilathangam G, Palaniappan N. Prevalence and phenotypic detection of erythromycin-induced resistance to clindamycin in MRSA isolates. *J Clin Diagn Res* 2011; **5**: 1195-1198.
[10] Dubey D, Rath S, Sahu MC, Patnaik L, Debata NK, Padhy RN. Surveillance of infection status of drug resistant *Staphylococcus aureus* in an Indian teaching hospital. *Asia Pac J Trop Dis* 2013; **3**: 133-142.
[11] Dubey D, Padhy RN. Antibacterial activity of *Lantana camara* L. against multidrug resistant pathogens from ICU patients of a teaching hospital. *J Herb Med* 2013; **3**: 65-75.
[12] Robredo B, Singh KV, Baquero F, Murray BE, Torres C. Vancomycin-resistant enterococci isolated from animals and food. *Int J Food Microbiol* 2000; **54**: 197-204.
[13] Dubey D, Padhy RN. Surveillance of multidrug resistance of two Gram-positive pathogenic bacteria in a teaching hospital and *in vitro* efficacy of 30 ethnomedicinal plants used by an aborigine of India. *Asia Pac J Trop Dis* 2012; **2**: 273-281.
[14] Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004; **39**: 309-317.
[15] Eisner A, Feierl G, Gorkiewicz G, Dieber F, Kessler HH, Marth E, et al. High prevalence of VanA-type vancomycin-resistant enterococci in austrian poultry. *Appl Environ Microbiol* 2005; **71**: 6407-6409.
[16] Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother* 2004; **54**: 321-332.
[17] Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R, et al. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J Antimicrob Chemother* 2004; **53**: 28-52.
[18] Tacconelli E, Cataldo MA. Vancomycin-resistant enterococci (VRE): transmission and control. *Int J Antimicrob Agent* 2008; **31**: 99-106.
[19] Mallik BK, Panda T, Padhy RN. Traditional herbal practices by the ethnic people of Kalahandi district of Odisha, India. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S988-S994.
[20] Shahcheraghi FM, Rahbar SM, Zahraei VS, Shooraj F. Transmission of *Vibrio cholera* O1 serotype inaba in a rural area of Qazvin, Iran associated with drinking water. *Asia J Epidemiol* 2009; **2**: 66-71.
[21] Souli M, Galani I, Giamarellou H. Emergence of extensively drug-resistant and pandrug-resistant Gram-negative bacilli in Europe. *Euro Surveill* 2008; **13**(47): pii: 19045.
[22] Mosquito S, Ruiz J, Pons MJ, Durand D, Barletta F, Ochoa TJ. Molecular mechanisms of antibiotic resistance in diarrhoeagenic *Escherichia coli* isolated from children. *Int J Antimicrob Agents* 2012; **40**: 544-548.
[23] Dubey D, Sarangi R, Debata NK, Padhy RN. Detection of metallo-β-lactamase producing *Klebsiella pneumoniae* in a neonatal septicemia. *J Acute Dis* 2013; **2**: 82-84.
[24] Valenzuela MT, O'Loughlin R, De La Hoz F, Gomez E, Constenla D, Sinha A, et al. The burden of pneumococcal disease among Latin American and Caribbean children: review of the evidence. *Rev Panam Salud Publica* 2009; **25**: 270-279.
[25] Quintero B, Araque M, van der Gaast-de Jongh C, Escalona F, Correa M, Morillo-Puente S, et al. Epidemiology of *Streptococcus pneumoniae* and *Staphylococcus aureus* colonization in healthy Venezuelan children. *Euro J Clin Microbiol Infect Dis* 2011; **30**: 7-19.
[26] Falagas ME, Rafailidis PI, Matthaiou DK, Virtzili S, Nikita D, Michalopoulos A. Pandrug-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections: characteristics and outcome in a series of 28 patients. *Int J Antimicrob Agents* 2008; **32**: 450-454.
[27] Sharma BC, Rai B. Incidence of multidrug resistance in *Escherichia coli* strains isolated from three lakes of tourist attraction (Mirik Lake, Jorepokhari Lake and Nakhapani Lake) of Darjeeling hills, India. *India J Fundam Appl Life Sci* 2012; **2**: 108-114.
[28] Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ, et al. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bull World Health Organ* 1999; **77**: 651-666.
[29] von Seidlein L, Kim DR, Ali M, Lee H, Wang X, Thiem VD, et al. A multicentre study of *Shigella diarrhoea* in six Asian countries: disease burden, clinical manifestations, and microbiology. *PLoS Med* 2006; **3**: e353.
[30] Wilson G, Easow JM, Mukhopadhyay C, Shivananda PG. Isolation and antimicrobial susceptibility of *Shigella* from patients with acute gastroenteritis in Western Nepal. *Indian J Med Res* 2006; **123**: 145-150.
[31] Nagano Y, Nagano N, Wachino J, Ishikawa K, Arakawa Y. Novel chimeric beta-lactamase C TX-M-64, a hybrid of C TX-M-15-like and C TX-M-14 beta-lactamases, found in a *Shigella sonnei* strain resistant to various oxyimino-cephalosporins, including ceftazidime. *Antimicrob Agents Chemother* 2009; **53**: 69-74.
[32] Uchida Y, Mochimaru T, Morokuma Y, Kiyosuke M, Fujise M, Eto F, et al. Geographic distribution of fluoroquinolone-resistant *Escherichia coli* strains in Asia. *Int J Antimicrob Agents* 2010; **35**: 387-391.
[33] Prats G, Mirelis B, Llovet T, Muñoz C, Miró E, Navarro F. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona. *Antimicrob Agent Chemother* 2000; **44**: 1140-1145.
[34] Taneja N, Lyngdoh V, Vermani A, Mohan B, Rao P, Singh M, et al. Re-emergence of multi-drug resistant *Shigella dysenteriae* with added resistance to ciprofloxacin in north India and their plasmid profiles. *Indian J Med Res* 2005; **122**: 348-354.
[35] Djie-Maletz A, Reither K, Danour S, Anyidoho L, Saad E, Danikuu F, et al. High rate of resistance to locally used antibiotics among enteric bacteria from children in Northern

Ghana. *J Antimicrob Chemother* 2008; **61**: 1315-1318.
[36] McMurry LM, Levy SB. The periplasmic protein MppA is not involved in regulation of *marA* in *Escherichia coli*. *Antimicrob Agents Chemother* 2011; doi: 10.1128/AAC.05030-11.
[37] Davin-Regli A, Bolla JM, James CE, Lavigne JP, Chevalier J, Garnotel E, et al. Membrane permeability and regulation of drug 'influx and efflux' in enterobacterial pathogens. *Curr Drug Targets* 2008; **9**: 750-759.
[38] Mamelli L, Petit S, Chevalier J, Giglione C, Lieutaud A, Meinnel T, et al. New antibiotic molecules: bypassing the membrane barrier of Gram negative bacteria increases the activity of peptide deformylase inhibitors. *PLoS One* 2009; doi: 10.1371/journal.pone.0006443.
[39] Pagès JM, James CE, Winterhalter M. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. *Nat Rev Microbiol* 2008; **6**: 893-903.
[40] Warnes SL, Highmore CJ, Keevil CW. Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: implications for public health. *mBio* 2012; doi: 10.1128/mBio.00489-12.
[41] Groisman EA, Ochman H. Pathogenicity islands: bacterial evolution in quantum leaps. *Cell* 1996; **87**: 791-794.
[42] Leekha S, Terrell CL, Edson RS. General principles of antimicrobial therapy. *Mayo Clin Proc* 2011; **86**: 156-167.
[43] Menrath A, Wieler LH, Heidemanns K, Semmler T, Fruth A, Kemper N. Shiga toxin producing *Escherichia coli*: identification of non-O157:H7-Super-Shedding cows and related risk factors. *Gut Pathog* 2010; doi: 10.1186/1757-4749-2-7.
[44] Rice LB. Mechanisms of resistance and clinical relevance of resistance to β-lactams, glycopeptides, and fluoroquinolones. *Mayo Clin Proc* 2012; **87**: 198-208.
[45] Sun PP, Perianayagam MC, Jaber BL. Endotoxin-binding affinity of sevelamer: a potential novel anti-inflammatory mechanism. *Kidney Int Suppl* 2009; doi: 10.1038/ki.2009.403.
[46] Sachdeva P, Misra R, Tyagi AK, Singh Y. The sigma-factors of *Mycobacterium tuberculosis*: regulation of the regulators. *FEBS J* 2010; **277**: 605-626.
[47] Bergval I, Kwok B, Schuitema A, Kremer K, van Soolingen D, Klatser P, et al. Pre-existing isoniazide resistance, but not the genotype of *Mycobacterium tuberculosis* drives rifampicin resistance codon preference *in vitro*. *PLoS One* 2012; doi: 10.1371/journal.pone.0029108.
[48] Alekshun MN, Levy SB. The mar regulon: multiple resistances to antibiotics and other toxic chemicals. *Trends Microbiol* 1999; **7**: 410-413.
[49] Ma D, Cook DN, Alberti M, Pon NG, Nikaido H, Hearst JE. Genes *acrA* and *acrB* encode a stress-induced efflux system of *Escherichia coli*. *Mol Microbiol* 1995; **16**: 45-55.
[50] Hagman KE, Shafer WM. Transcriptional control of the mtr efflux system of *Neisseria gonorrhoeae*. *J Bacteriol* 1995; **177**: 4162-4165.
[51] Jevons MP. ''Celbenin''-resistant staphylococci. *Br Med J* 1961; **1**: 124-125.
[52] Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. *Antimicrob Agents Chemother* 2004; **48**: 2637-2651.
[53] Murray BE. Beta-lactamase-producing enterococci. *Antimicrob Agents Chemother* 1992; **36**: 2355-2359.
[54] Uttley AC, Woodford N, Johnson AP, Cookson B, George RC, Wilcox M, et al. Vancomycin-resistant enterococci. *The Lancet* 1993; **342**: 615-617.
[55] Uttley AHC, Collins CH, Naidoo J, George RC. Vancomycin-resistant *Enterococci*. *Lancet* 1988; **1**: 57-58.
[56] Delahaye F, Hoen B, McFadden E, Roth O, de Gevigney G. Treatment and prevention of infective endocarditis. *Expert Opin Pharmacother* 2002; **3**: 131-145.
[57] Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* 1999; **43**: 2823-2830.
[58] Woodford N. Biological counterstrike: antibiotic resistance mechanisms of Gram-positive cocci. *Clin Microbiol Infect* 2005; **11**(Suppl 3): S2-S21.
[59] Sahu MC, Patnaik R, Padhy RN. *In vitro* combination efficacy of ceftriaxone and leaf extract of *Combretum albidum* G. Don against multidrug-resistant *Pseudomonas aeruginosa* and host-toxicity testing with lymphocytes from human cord blood. *J Acute Med* 2014; **4**: 26-37.
[60] Forbes BA, Sahm DF, Weissfeld AS. *Bailey and Scott's diagnostic microbiology*. 12th ed. Maryland Heights, Missouri: Mosby; 2007.
[61] Cockerill FR. CLSI M100-S21: performance standard for antimicrobial susceptibility testing: twenty-first informational supplement, M100S21. 2011.
[62] Sahu MC, Debata NK, Padhy RN. Antibacterial activity of *Argemone mexicana* L. against multidrug resistant *Pseudomonas aeruginosa*, isolated from clinical samples. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S800-S807.
[63] Panda T, Padhy RN. Ethnomedicinal plants used by tribes of Kalahandi district, Orissa. *Indian J Tradit Knowl* 2008; **7**: 242-249.
[64] Taylor SW, Alexander ME. Science, technology, and human factors in fire danger rating: the Canadian experience. *Int J Wildland Fire* 2005; **15**: 121-135.
[65] De Silva T, Centre for Science and Technology of the Non-Aligned and Other Developing Countries. *Traditional and alternative medicine: research and policy perspectives*. New Delhi: Daya Publishing House; 2009.
[66] Dubey D, Rath S, Sahu MC, Debata NK, Padhy RN. Antimicrobials of plant origin against multi-drug resistant bacteria including the TB bacterium and economics of plant-drugs-Introspection. *Indian J Tradit Knowl* 2012; **11**: 225-233.
[67] Altman RD. The future of herbal medicine in the treatment of osteoarthritis. *Osteoarthritis Cartilage* 2008; **16**: S10-S11.
[68] Lee J, Bielory L. Complementary and alternative interventions in atopic dermatitis. *Immunol Allergy Clin North Am* 2010; **30**: 411-424.
[69] Pineda MJ, Singh DK. What is integrative oncology and can it help my patients? *Obstet Gynecol Clin North Am* 2012; **39**: 285-312.
[70] Rath S, Padhy RN. Monitoring *in vitro* antibacterial efficacy of *Terminalia alata* Heyne ex. Roth, against MDR enteropathogenic bacteria isolated from clinical samples. *J Acute Med* 2013; **3**: 93-102.
[71] Nayak N, Rath SN, Mishra MP, Ghosh G, Padhy RN. Antibacterial potency of the fern terrestrial fern *Lygodium flexuosum* (L.) Sw. against multidrug resistant enteric-and uro-pathogenic bacteria. *J Acute Dis* 2013; **2**: 270-276.
[72] Rath S, Dubey D, Sahu MC, Debata NK, Padhy RN. Antibacterial activity of 25 medicinal plants used by aborigines of India against 6 uropathogens with surveillance of multidrug resistance. *Asian Pac J Trop Biomed* 2012; **2**: S846-S854.